

The present application is a continuation of U.S. Serial No. 08/851,162 filed May 5, 1997, which is a divisional of U.S. Serial No. 08/422,436 filed April 14, 1995, each of which is incorporated herein by reference in its entirety.--

In the Claims:

Please amend claims 1 and 22-25 to read as follows:

1. (Amended twice) A process for purifying correctly folded monomeric insulin-like growth factor-I (IGF-I) from a medium containing IGF-I peptides, comprising the steps of:

(a) contacting the medium with a sufficient quantity of a first cation exchange matrix under conditions allowing adsorption of at least about 95% of total IGF-I from the medium;

(b) washing the IGF-I-loaded first cation exchange matrix with a first cation exchange wash buffer, which removes adsorbed non-IGF-I material from said matrix and allows retention of authentic or non-authentic IGF-I by said matrix;

(c) eluting all forms of adsorbed IGF-I from the cation exchange matrix of step (a) by contacting said cation exchange matrix with a sufficient quantity of a first cation exchange elution buffer, which has a sufficiently high pH or ionic strength to displace substantially all of said authentic and non-authentic IGF-I from said cation exchange matrix;

(d) transferring the IGF-I-containing eluate from step (c) into an unfolding/refolding buffer, which:

(i) reduces the intrachain disulfide bonds of IGF-I protein and promotes unfolding without permanent denaturation; and

(ii) permits refolding of the IGF-I and reoxidation to form properly-paired intrachain disulfide bonds;

(e) contacting the properly folded IGF-I from step (d), after transfer into a suitable solvent system, with a sufficient quantity of a hydrophobic interaction chromatography matrix under conditions allowing adsorption of at least about 95% of said IGF-I from said eluate;

(f) washing the IGF-I-loaded hydrophobic interaction chromatography matrix with a hydrophobic interaction wash buffer having an ionic strength sufficiently low to remove most of the

non-authentic IGF-I from the hydrophobic interaction chromatography matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(g) eluting the adsorbed IGF-I from said hydrophobic interaction chromatography matrix by contacting said matrix with a hydrophobic interaction elution buffer, which has a sufficiently elevated pH, or sufficiently low ionic strength, to cause displacement of substantially all of the adsorbed authentic IGF-I from said matrix;

(h) contacting the eluate from step (g) with a sufficient quantity of a second cation exchange matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(i) washing the IGF-I-loaded second cation exchange matrix with a cation exchange wash buffer having a sufficiently high ionic strength, or sufficiently high pH, to remove non-authentic IGF-I from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix;

(j) eluting the adsorbed IGF-I from said second cation exchange matrix by contacting said matrix with a second cation exchange elution buffer, which has a sufficiently high ionic strength, or sufficiently high pH, to displace substantially all of the adsorbed authentic IGF-I from said matrix;

(k) contacting the eluate from step (j), in an aqueous buffer, with a suitable quantity of a reverse phase chromatography matrix under conditions allowing adsorption of at least about 95% of the IGF-I from the eluate;

(l) washing the IGF-I-loaded reverse phase chromatography matrix with an aqueous/organic reverse phase wash buffer having an organic solvent concentration sufficiently high to remove non-authentic IGF-I from said matrix while retaining substantially all of the adsorbed authentic IGF-I on said matrix; and

(m) eluting the adsorbed IGF-I from said reverse phase chromatography matrix with an aqueous/organic buffer having an organic solvent concentration high enough to remove substantially all of the authentic IGF-I without removing substantially all of the multimeric forms of IGF-I from said matrix.